



## Development of a Transgenic Model to Assess Bioavailable Genotoxicity in Sediments

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**PURPOSE:** This technical note describes the rationale for using transgenic animal models to assess the potential genotoxicity of sediments, the benefits that can be obtained using such models versus currently used methods, the nature of the transgenic model, and the modification of an existing transgenic construct being used to develop a mutation model based on a polychaete worm at the U.S. Army Engineer Research and Development Center (ERDC). The research described in this technical note was conducted with support from the U.S. Army Engineer Long-Term Effects of Dredging Operations (LEDO) Program.

**BACKGROUND:** Many of the contaminants (metals as well as organics) commonly encountered in dredged sediments are genotoxic, i.e., capable of damaging genetic material. Open ocean disposal of genotoxins other than trace contaminants is expressly prohibited in the regulations governing ocean disposal of dredged sediments. Specifically, Section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972 (MPRSA) at § 227.6 (a) (5) prohibits "known carcinogens, mutagens, or teratogens or materials suspected to be carcinogens, mutagens, or teratogens by responsible scientific opinion," "as other than trace contaminants" if there is a reason to believe such chemicals are present. Section 103 defines "trace contaminants" as constituents present "in such forms and amounts...that the dumping of the materials will not cause significant undesirable effects...in marine organisms" (§ 227.6 (b)). The potential for "significant undesirable effects" must be determined by bioassays (§ 227.6 (c)), and "material shall be deemed acceptable for ocean dumping" when "bioassay results on the solid phase of the wastes do not indicate occurrence of significant mortality or significant adverse sublethal effects due to the dumping of wastes containing the constituents listed in paragraph (a) of this section" (§227.6 (c) (3)). However, the recommended tests that are ordinarily performed in dredged sediment regulation (bioaccumulation and acute toxicity tests) are incapable of detecting genotoxicity. This technical note presents an approach that may be used to assess potential biologically available genotoxicity of contaminated sediments.

Part of the problem in determining genotoxicity is that the classical endpoint for carcinogenicity investigations is the detection of neoplasms (tumors). While various short-term assays are used to identify presumptive carcinogens, the assays employed in the regulatory testing of new pharmaceutical, industrial, and agricultural chemicals involve detection of neoplasms in a chronically exposed mammalian species, usually rats. Although tumors in native benthic fish populations have been used as in vivo biomarkers of genotoxicant exposure (Baumann and Harshbarger 1985, 1995, 1998; Baumann et al. 1990; Malins et al. 1985; Myers et al. 1987), this endpoint has little use for testing dredged sediments. The time requirements (near life span) and the large numbers of organisms necessary to test for the development of tumors are insurmountable limitations for using tumorigenesis in dredged sediment testing.

Investigations have been ongoing at ERDC for several years into methods that may be adaptable for testing the genotoxicity of dredged sediments. Results of this work show that while many in vitro

assays for genotoxicity are available, very few in vivo assays exist that can be applied to sediment-dwelling organisms. The in vitro assays have numerous limitations, not the least of which is the necessity of testing sediment extracts. The actual fractions of genotoxic chemicals in sediments that are bioavailable to organisms are not represented by extracts, cannot be predicted, and can only be determined in bioassays involving exposure of appropriate organisms directly to the sediments. While capable of discriminating the bioavailable fraction of genotoxins, the in vivo assays that do exist also have severe limitations. In some cases, the type of DNA damage they identify is insufficient to determine real probability of eventual development of cancer or of developmental abnormalities, or the assays must rely on field-exposed animals, or the animal is not intimately associated with sediments in its normal habitat. This technical note describes an approach that applies new advances in biotechnology to address the shortcomings in existing sediment genotoxicity testing.

**INTRODUCTION:** At the present time, the genotoxicity tests that are most commonly applied to sediments are in vitro bacterial assays such as Mutatox, Ames assay, SOS chromotest and others (Ho and Quinn 1993, Jarvis et al. 1996, Johnson 1992, Legault et al. 1994) in which bacterial mutations resulting from exposure to sediment extracts are quantified. However, as these tests are based on extracts, they measure the presence of mutagens but do not reflect their bioavailability, and are thus not interpretable in terms of either ecological or human risk assessment. Additionally, the exact relevance of mutations occurring in microbes to the potential for genotoxic effects in wildlife or humans is unknown. These limitations also apply to the various cell lines (yeast, fish, mammals) that can be used for detection of genotoxic compounds (Gagné et al. 1995, Kocan et al. 1985, Mueller et al. 1991, West et al. 1986a, 1986b).

Some short-term in vivo assays for genotoxicity have been adapted for aquatic organisms. Most of the available assays detect damage to DNA, including assays for DNA strand breaks, e.g., alkaline unwinding (Everaarts 1995, Shugart 1988a, 1988b), alkaline filter elution (Vukmirovic et al. 1994), the Comet assay (Deventer 1996, Pandrangi et al. 1995) or chromosomal aberrations, e.g., sister chromosome exchange (SCE) (Dixon and Clarke 1982, Pesch et al. 1981, 1985), aneuploidy (Dixon 1982), micronuclei formation (Dopp et al. 1996, Weis et al. 1995), and total chromosomal aberrations (Baksi and Means 1988, Jha et al. 1996, Means and Daniels 1988). Unfortunately, only a few have been adapted to use in animals appropriate for sediment testing; e.g., SCEs in *Neanthes* (Pesch et al. 1981, 1985), chromosomal aberrations in *Platyneris* (Jha et al. 1996). These tests monitor only gross alterations in the DNA superstructure and cannot detect more subtle genetic damage such as point mutations.

<sup>32</sup>P-postlabelling can detect adducts on DNA, which can potentially result in single strand breaks and point mutations, and correlates well with pathological responses in mussels (Kurelec et al. 1990). However, <sup>32</sup>P-postlabelling requires the use of one of the more hazardous radiotracers, and can only detect damage caused by a select group of chemicals (primarily aromatic hydrocarbons). Additionally, the utility of this method in monitoring environmental exposure to genotoxins has been questioned, primarily due to a high rate of false negatives (non-detection of genotoxic contaminants) potentially caused by DNA repair processes and inconsistent responses between different species (Lloyd-Jones 1995).

Fluorescent aromatic compounds (FACs) in bile of exposed fish have been used to monitor potential sediment genotoxicity (Collier and Varanasi 1991, Johnston and Baumann 1989), but like

<sup>32</sup>P-postlabelling, this assay is limited to detection of aromatic hydrocarbons. One promising assay measures alterations in the *ras* oncogene in medaka (Torten et al. 1996), but this fish is a freshwater top minnow native to rice ponds and is unsuitable for testing dredged sediments.

**INTERPRETING DNA DAMAGE:** There is a strong correlation between the ability of a chemical to cause mutations and its ability to cause cancer, but merely detecting the ability of a compound to cause mutations does not provide sufficient information for making reasoned judgements regarding the potential for cancer or for other genetically determined toxicities. Mutations have a basal rate of occurrence in all organisms, and repair and compensatory mechanisms exist that normally maintain the organism's health. It is only when the rate of mutation exceeds the organism's ability to repair the damage that the probabilities of adverse effects increase. The principal reason for assessing mutation is that it is the earliest detectable link in the chain of events that lead to tumor formation. Carcinogenesis is generally considered to be a result of multiple events (Pitot et al. 1988). In a simplified version of the most common multistage carcinogenesis theory, the first event, initiation, results in irreversible DNA damage (the cell must divide after a mutation has occurred in order to "fix" the alteration before the damage is repaired). This is followed by a second, reversible stage (promotion), in which the cell undergoes several divisions to form a population of altered cells (a foci). The final stage, progression, occurs when the foci undergoes a final irreversible alteration, resulting in a population of cells with measurable or morphological changes in the cell genome (a tumor).

Knowledge of the nature of the mutations is critical to interpretation of the potential effects of the mutation. Mutagens that primarily cause base deletions that result in a frameshift of the DNA can result in nonsense coding that is disastrous to the ability of a gene to encode an essential enzyme or other protein, and can lead to the death of the mutated cell. While this compound may not be carcinogenic, it could be disastrous in a developing embryo where the death of a single cell at a critical time and place may result in severe birth defects (teratogenesis). Large base deletions are also known to result in activation of oncogenes, which are genes that when activated contribute to the emergence of malignant tumors (Paul 1988). More subtle mutations such as point mutations have also been shown to activate oncogenes. Thus, assays that determine what type of DNA damage is occurring greatly improve the ability to predict the potential effects. Additionally, if it is possible to identify the specific sites of mutation in a target gene in response to a complex mixture such as dredged sediments, a mutation spectrum can be described that can be compared to the mutation spectra for specific chemicals in numerous databases that have been compiled and are available on the Internet. For example, the *lacI* database for mutants recovered from Stratagene's Big Blue® rodents and cell lines contains information on the position of the mutations, the actual changes observed, the mutagen responsible, the tissue the mutations are observed in, the dose causing the mutations, the animal strain used, literature references, etc. (University of Victoria, BC 1998). With this information, it may be possible to determine the class or classes of contaminants in the sediment most likely responsible for the genotoxic effect, the probable dose to the organism, and the probability of the incidence of cancer or developmental abnormalities in the exposed population. This is the type of information required for realistic risk assessment. The ability to identify mutation spectra represents a major advance in the interpretability of mutation that has only been made possible by the advent of transgenic animal models such as Big Blue® rodents.

**TRANSGENIC MODELS FOR MUTAGENESIS:** A transgenic animal is one that has had foreign DNA (a transgene) inserted into its genome. Several strains of transgenic animals have been developed for testing mutagens in whole animals, including the Big Blue® mouse and rat and the Muta<sup>TM</sup>Mouse. In these animals, the transgene is made up of two essential parts: (1) the *reporter gene* that serves as a target for mutation, and (2) a *shuttle vector* for recovering the reporter gene DNA from the tissue of the transgenic animal.

- **A Color Screening Assay: the *lacI/lacZ* Reporter Gene-Based System:** Several reporter genes are presently used in in vivo genotoxicity testing in mammalian models. The best-characterized system is BigBlue®, which employs the microbial *lacI/lacZ* genes in a lambda phage shuttle vector as a mutational target inserted into the genome of a mouse and a rat. When the rodent is exposed to a mutagen, the DNA of the reporter gene is damaged along with the DNA of the rodents' genome. The animal is then sacrificed and the high-molecular-weight DNA is isolated from the target tissue. The DNA containing the reporter gene is then packaged into a viable bacteriophage (a virus that infects bacteria) using Transpack<sup>TM</sup> in vitro packaging extract, a mixture of bacteriophage proteins, enzymes, and other reagents. The bacteriophage is plated with a bacterial host culture on top agar that contains a chromogenic substance, X-gal. The phages now containing the reporter gene infect some of the bacteria in the culture. The mixture of infected and uninfected bacteria is allowed to grow, forming a "lawn" of bacteria. Bacteriophage-infected cells expressing the lambda phage genes eventually cause the cell to lyse and release active phages into the nearby agar. As more and more nearby bacteria are infected and lyse, a hole or "plaque" forms on the bacterial lawn.

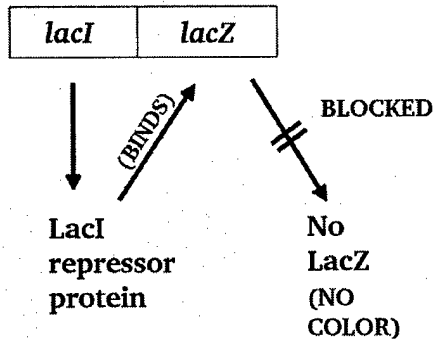
In an undamaged *lacI* reporter gene, gene expression occurs and results in the transcription of the LacI repressor protein. This protein binds to the operator region of the *lacZ* gene and suppresses transcription of that gene. Without the LacZ protein, the enzyme  $\beta$ -galactosidase ( $\beta$ -Gal) is not active (Figure 1), the bacteria are unable to cleave the chromogenic substrate X-gal that is incorporated in the agar, and the plaques that eventually form remain clear. On the other hand, if a mutation has occurred in the *lacI* gene, the *lacZ* gene will not be suppressed. The LacZ protein combines with the carboxy terminus of the enzyme (produced by the bacterial host cell), forming the active  $\beta$ -Gal enzyme. The enzyme will cleave the X-gal, releasing a product that gives a blue coloration to the plaques that eventually form. Thus, damage to the DNA in the *lacI/lacZ* reporter gene system can be quantified as the mutant ratio, or the ratio of blue plaques (mutant reporter genes) to colorless plaques (non-mutated reporter genes). Additionally, the exact mutation foci can be determined after isolating the blue plaques and amplifying the *lacI* DNA sequence of the phage particles using polymerase chain reaction (PCR) after restriction endonuclease isolation of the *lacI* sequence. The patterns of mutation foci are the mutation spectra that can be compared against existing databases.

This system has been used successfully in several organisms, and the gene and its mutation spectra have been well characterized. However, there are problems with the assay, a key one being the low efficiency of recovery. Using the Transpack<sup>TM</sup> in vitro packaging extract, only about 3 percent of the theoretical maximum recoverable transgenes present in the tissue are packaged into the bacteriophage (Gossen et al. 1993). Due to the low efficiency, large numbers of agar plates are required to observe enough plaques for statistical analysis and calculation of the rate of mutation. As the bacterial lawn on a standard agar plate cannot contain many

## THE *lacI/lacZ* SYSTEM

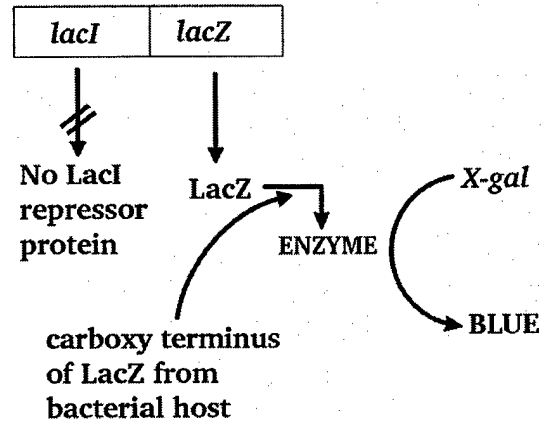
### NORMAL GENE

- *LacI* repressor protein blocks *LacZ* production
- COLORLESS PLAQUE



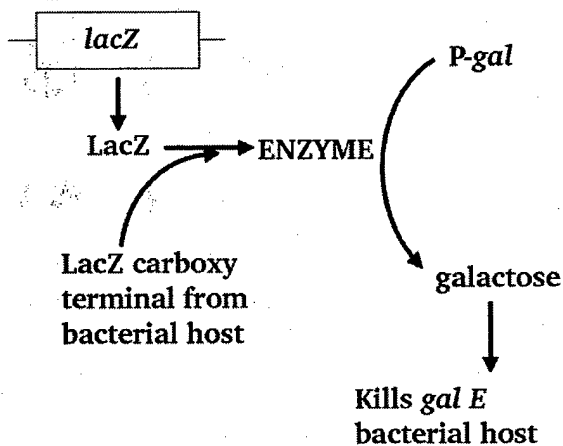
### MUTANT GENE

- *LacI* repressor protein inactive
- PLAQUES ARE BLUE



## THE *lacZ* SYSTEM

- NORMAL GENE - *LacZ* produced  
- No Growth  
- NO PLAQUE



- MUTANT GENE - *LacZ* non-functional  
- bacterial growth/phage reproduction  
- CLEAR PLAQUE

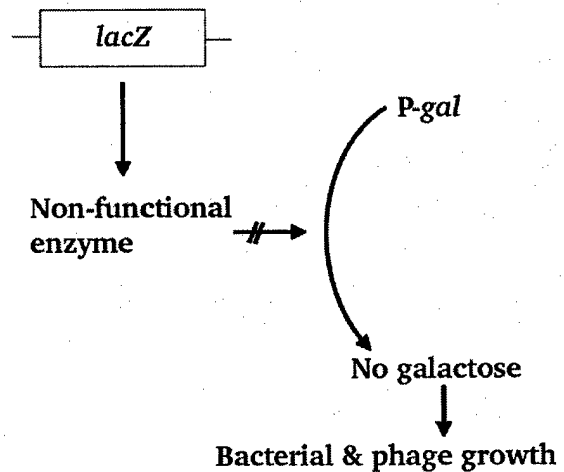


Figure 1. *lacI/lacZ* and *lacZ* reporter systems

plaques before they begin to merge, special large plates must be used (Figure 2). These are expensive, difficult to pour agar into evenly, and even more difficult to keep sterile (molds and airborne bacteria contaminate the plates, rendering them useless).

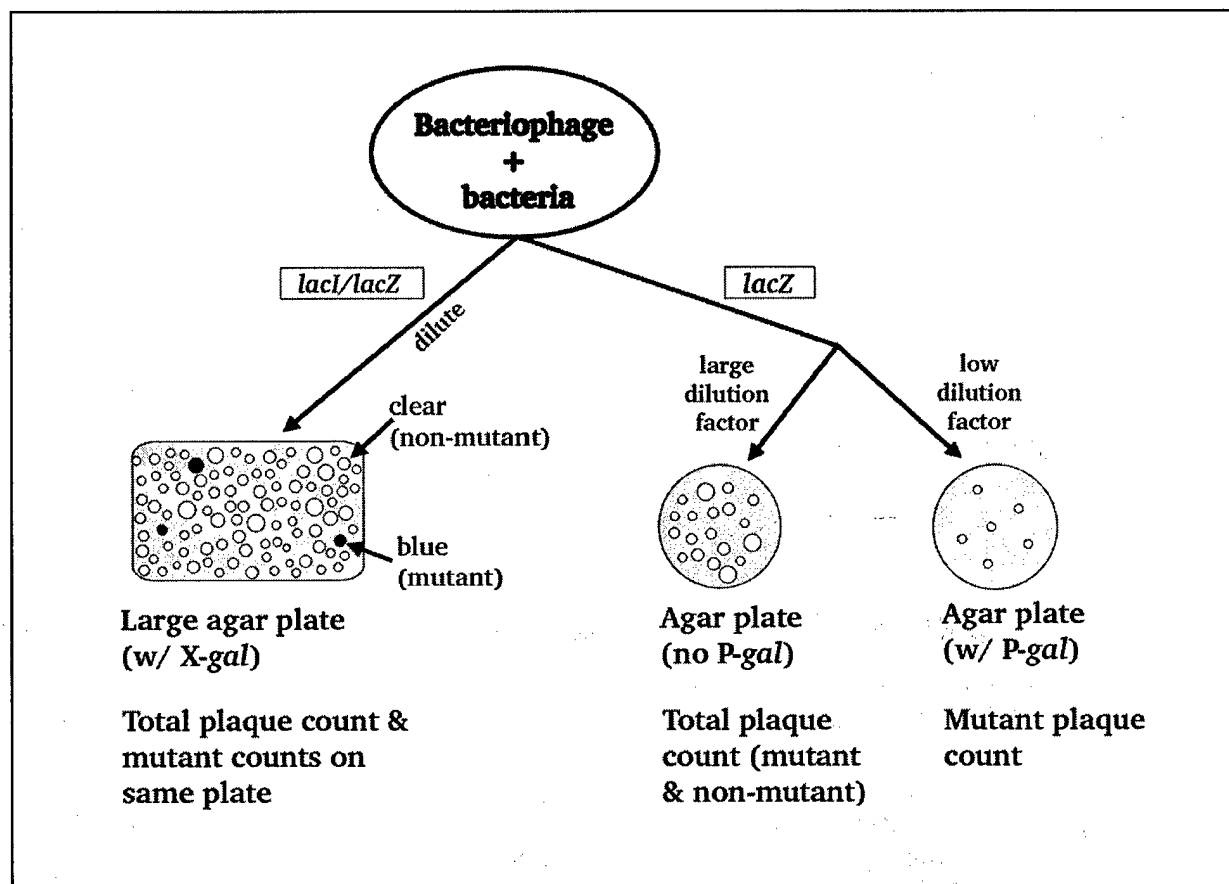


Figure 2. Comparison of the *lacI/lacZ* and *lacZ* lambda phage detection systems

- A Selection Assay: the *lacZ* Reporter Gene Based System:** Muta<sup>TM</sup>Mouse is an alternative transgene system also based on a lambda phage shuttle vector and the Transpack<sup>®</sup> in vitro packaging extract system. However, the Muta<sup>TM</sup>Mouse contains only the *lacZ* gene in the shuttle vector. In the non-mutated reporter gene, the *LacZ* protein is synthesized and active  $\beta$ -Gal is formed. The enzyme cleaves the substrate P-gal (incorporated in the agar upon which the bacteria were plated), releasing galactose. The galactose prevents both the host bacteria and the bacteriophage from replicating (Figure 1). A mutation on the reporter gene at the *lacZ* site will result in a nonfunctional  $\beta$ -Gal, allowing the bacteria and the bacteriophage to replicate. Again, the bacteriophage eventually replicate to the point where the host cell dies, forming a clear plaque. Thus, in the *lacZ* reporter gene system, a normal reporter gene will prevent the plaque from forming, while the mutated gene will form a clear plaque on the bacterial lawn. In order to obtain the mutant ratio, a second plate is made without the P-gal to obtain the total number of plaques. The advantage of this procedure is the reduction in the number of agar plates required. Unlike the *lacI/lacZ* reporter gene system, only the mutant reporter genes will form plaques in the presence of P-gal, and the samples can be plated with

concentrated bacteria/ bacteriophage mixtures without fear that the plaques will merge and prevent accurate counting (Figure 2). Thus, a smaller number of standard-sized agar plates is required. However, the assay retains the problem of low packaging efficiency of the Transpack™ in vitro packaging extract system.

- **A Plasmid Recovery-Based System:** The Transpack™ packaging efficiency problem is solved by a new method for transgene recovery, based on a plasmid rather than a bacteriophage (Boerrigter et al. 1995, Gossen et al. 1993). Plasmids are circular duplex DNA molecules that can carry genes for a variety of functions, and act as accessory chromosomes. They can replicate independently of the host and are distinct from bacterial chromosomes, which are replicated by the cell. When using the plasmids as shuttle vectors, the reporter gene is inserted into the plasmid, which is then linearized by opening the circular plasmid with the restriction enzyme *Hind III*. The linearized plasmid is then inserted into the genome of an organism to form the transgenic animal in the same manner one would generate a transgenic animal using a bacteriophage shuttle vector. The reporter gene is recovered from the genomic DNA of the organism by isolating the DNA from the target tissue and digesting it with *Hind III* (Figure 3). The clipped-out transgene is then purified from the genomic DNA using LacI repressor protein bound to magnetic beads. The LacI repressor protein binds to the *lacZ* gene, which can then be rinsed free of unwanted DNA fragments and pelleted on a magnetic stand. The plasmid is then ligated (recircularized), eluted from the magnetic beads with IPTG (isopropylthio- $\beta$ -galactoside), and electroporated into the bacterial test strain. Electroporation is a procedure in which the bacterial cells are exposed to an exponentially decaying electrical pulse, causing the cell membrane to form pores through which the plasmid is able to pass. Plasmid-based transgene recovery systems result in much higher recoveries: 70 to 80 percent of the theoretical maximum number of transgenes, as compared to 3 percent obtained by packaging into bacteriophages (Boerrigter et al. 1995). Another advantage of this system is that plasmid DNA recovery has no DNA fragment size limitations, unlike the bacteriophage systems, which require that the DNA being recovered is <42 kb or >52 kb in size. This allows the plasmid system to not only detect point mutations but also large DNA deletions and additions, which the bacteriophage shuttle vector may miss.
- **Detection of Mutants in Plasmids:** Mutant detection in the plasmid recovery system is different than in bacteriophage recovery (Figure 4) because plasmids will not cause plaque formation. In order to determine the total recovered plasmids, a portion of the bacteria that have been electroporated with the recovered plasmids are allowed to grow on agar plates that contain ampicillin and X-gal. The ampicillin will allow only bacteria containing the plasmid to survive, since the host bacterial culture is ampicillin sensitive and the plasmid pUR288 contains an ampicillin-resistance gene. The survivors will form blue colonies of bacteria on the plates, which can be easily counted to determine the total number of recovered plasmids. To determine the number of mutant plasmids, another (less dilute) portion of the electroporated bacteria is allowed to grow on plates containing ampicillin and P-gal. Only mutant bacteria will grow on this plate; bacteria harboring the normal plasmid will not grow because the normal LacZ protein allows formation of an active  $\beta$ -Gal enzyme, releasing galactose from the P-gal and killing the bacteria. Bacteria that did not successfully incorporate the plasmid during electroporation will not grow either, due to their sensitivity to ampicillin.

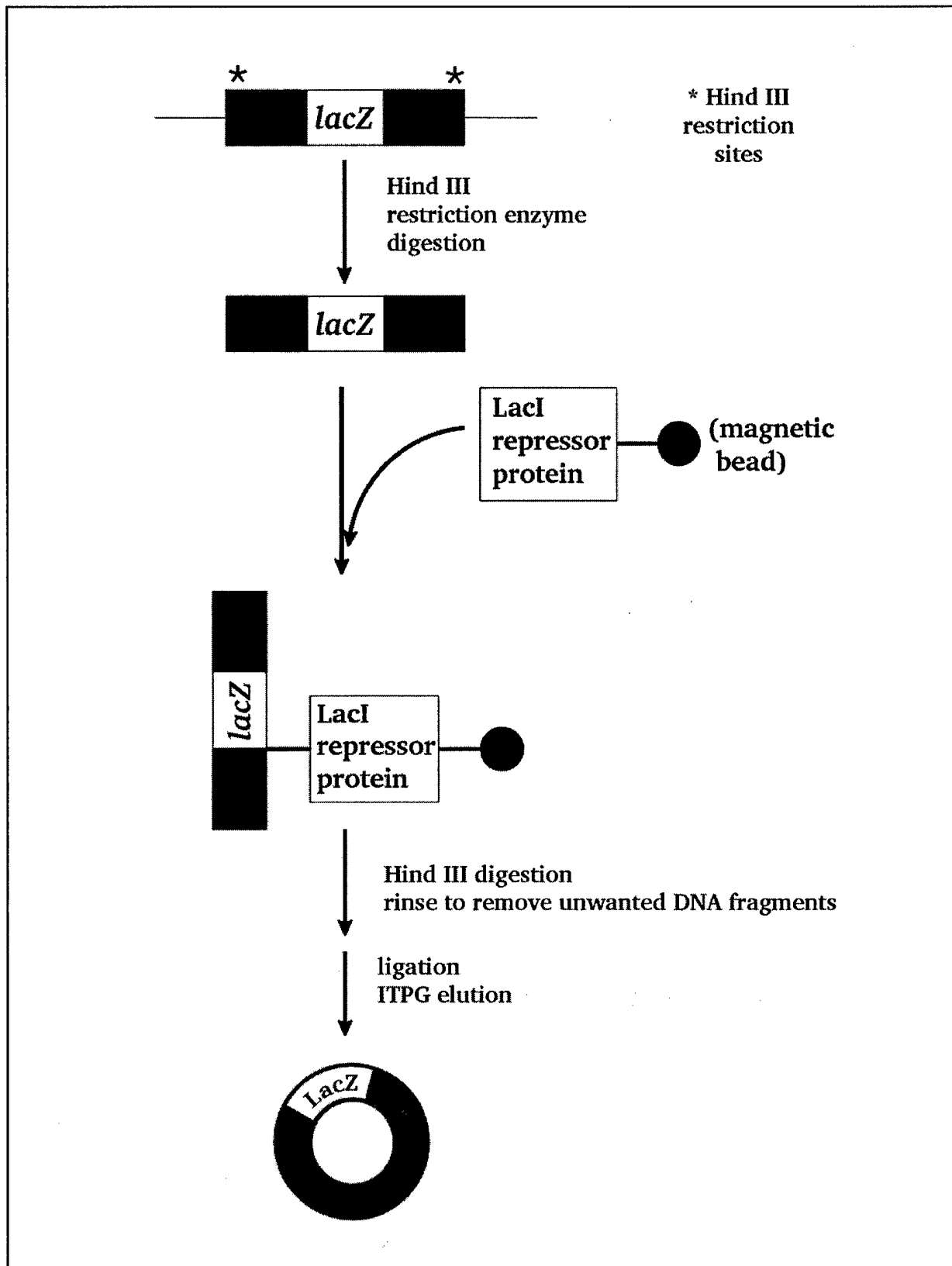


Figure 3. Plasmid rescue: removal and isolation of the plasmid reporter gene from the transgenic animal

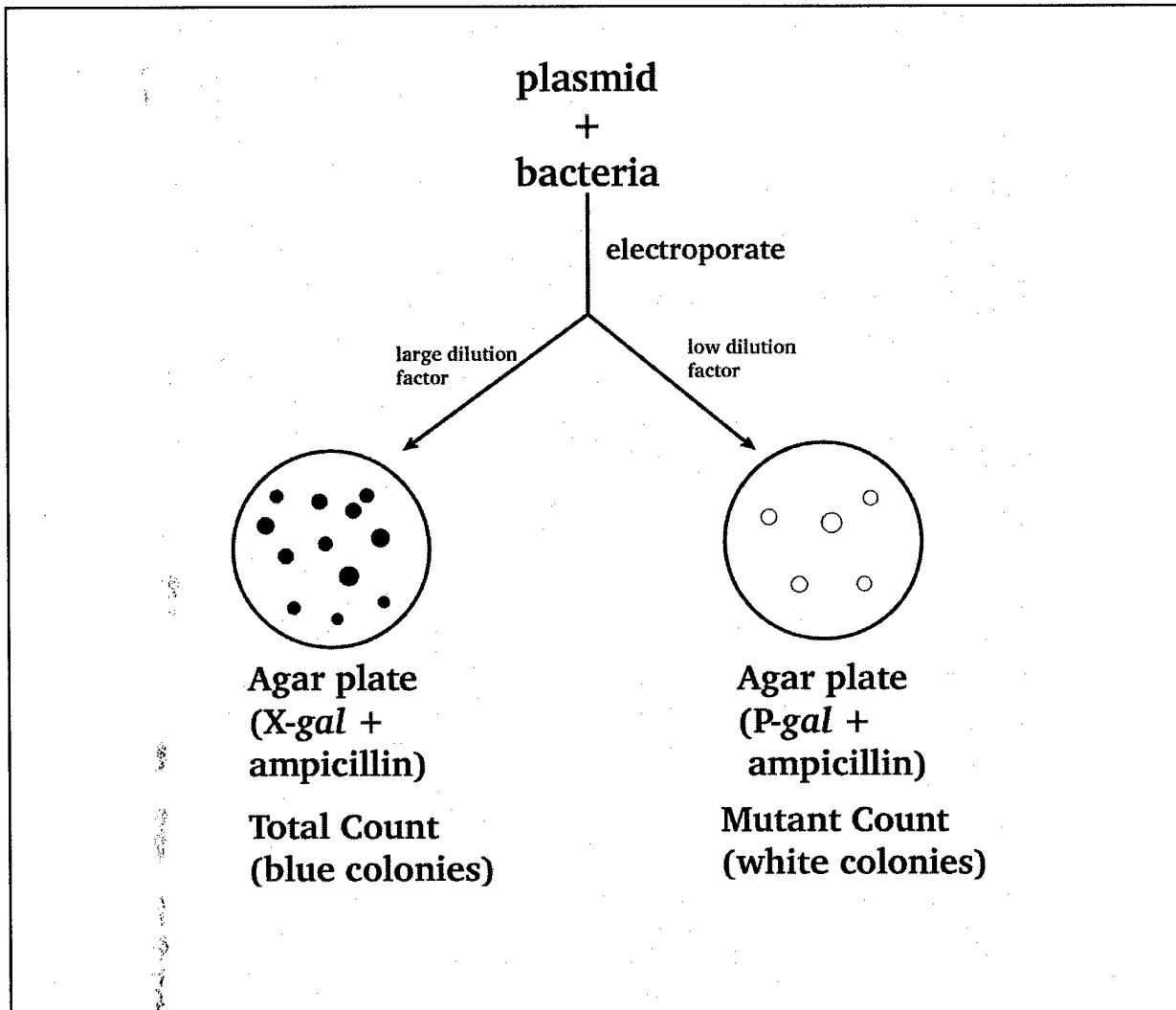


Figure 4. The plasmid detection system

**TRANSFECTION TECHNIQUES:** Once the reporter and marker genes have been selected, they must be incorporated into the organism that has been selected (transfection). It is highly desirable to have the genes present in all cells of the organism. If an egg is transfected after it has been fertilized and begun to divide, it will develop into a chimera, i.e., an organism in which some cells contain the transgene, but other cells do not. Therefore, it is preferable to transfect the gametes prior to fertilization. The genes can be inserted into the gametes via several methods but the two most commonly used methods are microinjection and electroporation. In the microinjection technique, the DNA is injected into the egg using a microscopic needle, and the egg is then fertilized. The technique works well, although many eggs fail to develop properly due to damage resulting from being punctured by a needle (about 10 percent of the injected eggs successfully develop).

Electroporation is faster (many eggs can be transfected at one time, versus microinjecting one egg at a time) and easier for smaller eggs ( $<100\ \mu\text{m}$ ). The most effective voltages for the electroporation of eggs yield about a 50-percent survival rate. Electroporation can also be applied to sperm; electroporated sperm retain high viability with successful fertilization rates of 94.7-99.6 percent, which

allows transformation of large numbers of eggs at one time with transgenic embryo production as high as 65 percent (Tsai et al. 1997). Facilities available at ERDC permit experimentation with both of these procedures.

Once the genes have been inserted into the gametes, they are fertilized and allowed to develop into the first generation of the transgenic organism (F1). In order to assure that the reporter gene is stable in the organism, the F1 generation is then cross-bred to generate a stable transgenic line, whose DNA is analyzed using PCR and gene sequencing to confirm the presence of the reporter gene. If the gene construct has successfully been incorporated into the organism's genome, the response of the transgenic animals to standard mutagens and carcinogens is then characterized. After this stage, the transgenic line is ready for field testing.

**ERDC TRANSGENE SYSTEM:** The transgene system under development at ERDC will combine the best elements of the systems described above. The plasmid-rescue vector pUR288 (Figure 5A) (Gossen et al. 1993) combines the advantages of the *lacZ* reporter gene and the plasmid recovery method, resulting in an easy-to-score assay and good transgene recovery.

- **Addition of a Modified Marker Vector:** The ERDC model will also include a cotransformation marker vector that will allow visual determination of whether or not a developing organism has been successfully transfected. Unlike a reporter gene, which is not actively expressed in the animal and is present to function as a target for mutagens, a marker gene is under the control of an active promoter gene, causing expression of a marker protein. Typically a 1:10 to 1:100 ratio of marker vector to reporter vector is used during transfection in order to increase the likelihood that a cell containing the marker vector also carries the reporter vector. The ERDC marker gene will be a modification of a commercially available cotransformation vector, pHygEGFP (Figure 5B) (Clontech Laboratories, Inc., Palo Alto, CA). This vector contains a fusion of the hygromycin resistance gene with the enhanced green fluorescent protein (EGFP) gene, which is expressed under control of the strong immediate early promoter of human cytomegalovirus (CMV). Expression of the fusion protein (Hyg-EGFP) will cause successfully transformed organisms to fluoresce when exposed to the proper light wavelength, providing a visual marker of successful transfection. Additionally, the hyg-EGFP fusion protein will allow selection of transfectants by hygromycin resistance. However, if transgenic organisms are created using pUR288 and pHygEGFP, both plasmids have ampicillin resistance and both would be recovered during ampicillin selection and falsely elevate the total recovered plasmid count (Figure 4). Therefore, for use as a cotransfection marker with pUR288, the Hyg/EGFP fusion will be cloned into a vector backbone without ampicillin resistance, pZER0 (Figure 5C). If the initial attempts at transfection using this construct prove successful, they will be continued. If, however, the human CMV promoter proves unsuitable for expressing the Hyg/EGFP fusion protein in invertebrate cells, an alternative vector is being prepared in which the CMV promoter is replaced with the drosophila (fruit fly) Actin 5c promoter from pPacO (Figure 5D).

**SPECIES SELECTION:** In selecting an appropriate species to develop as a transgenic model for assessing bioavailable genotoxicity in sediments, critical considerations are habitat, dietary preferences, life cycle, adult size, suitability for rearing in the laboratory, and ability of the organism to metabolically activate potential carcinogens. An essential requirement is that the organism be benthic

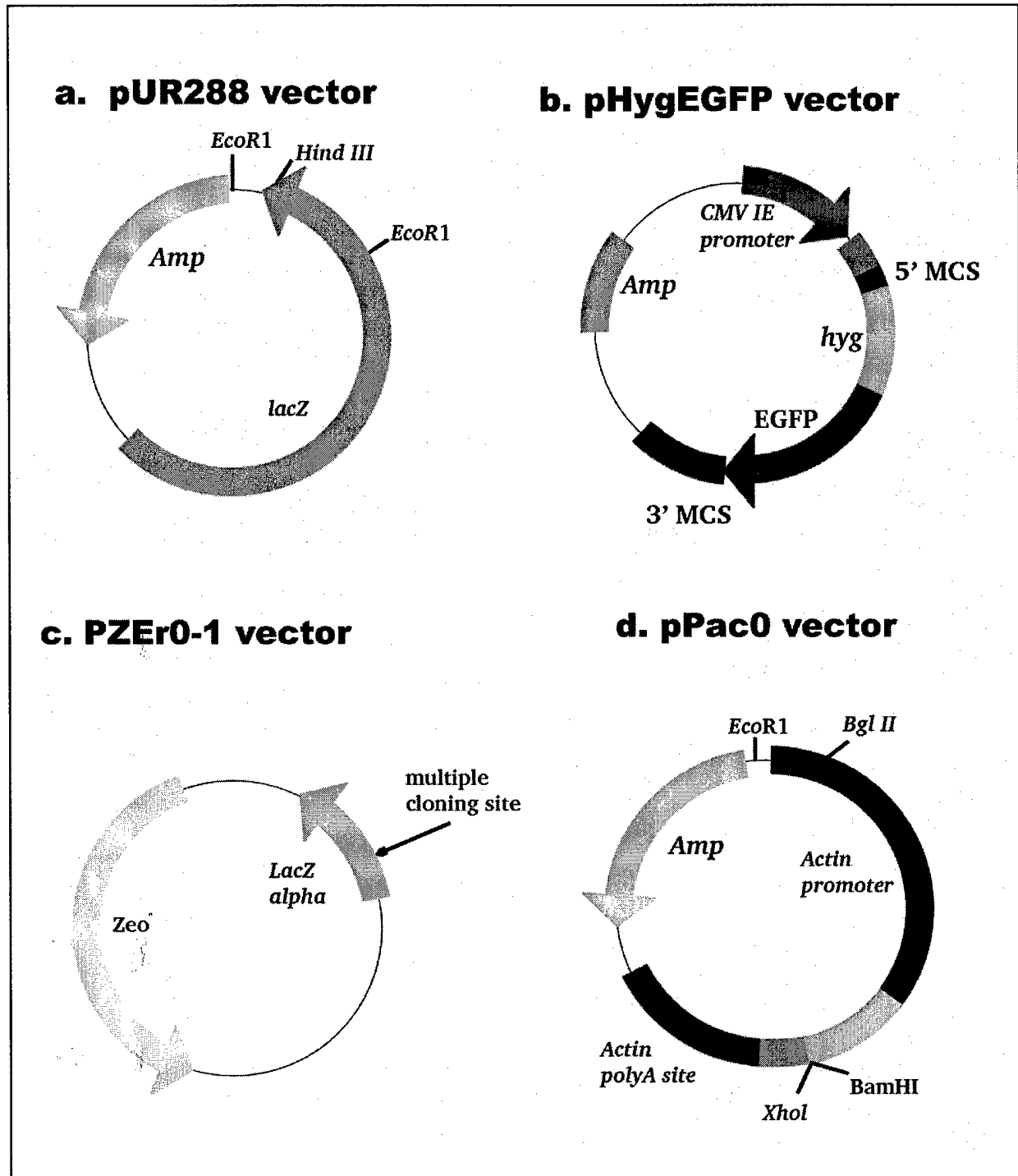


Figure 5. Plasmid vector maps: Restriction sites: *EcoR*I, *Hind* III, *Bgl* II, *Xho*I, *Bam* HI; Resistance genes: *Amp*<sup>r</sup>, *Zeo*<sup>r</sup>, *Hyg*<sup>r</sup>; Reporter gene: *lacZ*; Marker gene: *HygEGFP* promoters: *CMV* 1E, *actin* promoter. A) The pUR288 plasmid. B) The pHygEGFP plasmid vector. C) The PZEr0-1 plasmid vector; the *hyg/EGFP* genes from the pHygEGFP will be spliced into the multiple cloning site. D) The pPac0 plasmid vector; if the *CMV* promoter does not function in an invertebrate system, the *Hyg/EGFP* will be put under control of the *actin* promoter

infaunal, i.e., in intimate contact with the sediment in its normal habitat. A major criticism of the whole-organism bioassays presently used for genotoxicity is that the organisms are usually not in intimate and continuous contact with the exposure medium, the sediment.

Dietary intake plays a major role in both bioaccumulation and toxicity of hydrophobic contaminants present in sediments (Bridges et al. 1997, Forbes et al. 1998), while exposure to pore water plays a lesser role. Thus, it is desirable that the organism feed on sedimentary organic carbon rather than algae or other food sources. The life cycle of the organism also presents many restrictions, the most important being that the species must be free spawners (release the eggs and sperm directly into the water column) rather than being brooders (eggs cared for by the adults) or having internal fertilization. This is important, as the eggs or sperm must be obtainable so that they can be transfected with the reporter gene before fertilization takes place. The selected species must be amenable to laboratory culture, with a rapid rate of maturation so that it does not require several years just to establish a stable transgenic line. Adults should be of sufficient mass that there is enough tissue to extract the transgene for the mutagenicity assay, but not be so large that large amounts of space are required to maintain them or run exposures. The last and extremely important requirement is that the organisms be capable of biotransforming polycyclic aromatic hydrocarbons (such as benzo[a]pyrene) into their carcinogenic metabolites. Polycyclic aromatic hydrocarbons (PAHs) are an important class of carcinogenic sediment contaminants, and must be activated through a specific route of biotransformation. A transgenic organism that does not biotransform these compounds, or biotransforms them through routes that do not result in the carcinogenic metabolites, will not detect this major class of sediment carcinogens as being genotoxic.

*Marine Polychaetes:* Due to their habitat preferences, marine polychaetes are well-suited for sediment toxicity testing, which is why they are among the recommended species in the "Green Book," the testing manual for ocean disposal of dredged material for determining potential benthic impact (USEPA/USACE 1991). Additionally, many are free-spawning in their reproductive strategy, producing large quantities of gametes. Other species listed in the manual include several amphipods and crustaceans, but these tend not to be free-spawning species. Spawning can be induced by controlling temperature and light conditions, and many species spawn at multiple times during the year. Marine polychaete worms also satisfy most of the other requirements for selection as transgenic mutagenicity models listed above. Many polychaete species have been reared for multiple generations in the laboratory and much is known about their life histories and requirements for survival and reproduction. The adults of most species live in close contact with sediments, often process the sediments by ingestion, and are tolerant of fine-grained material.

The polychaete initially selected as a candidate for the basis of a transgenic mutation model at ERDC was *Nereis grubei*. However, while the species is benthic and free-spawning, their preferred food is algae, not detritus. Another limitation is that they live in mucus tubes on the surface of the sediment, rather than burrowing into the sediment, thus reducing their exposure to sediment-bound contaminants. The major limitation on which this initial species choice was rejected was that when exposed to [<sup>14</sup>C]-benzo[a]pyrene, no radiolabel was found to be bound with the worms' DNA, indicating that they did not activate PAHs to carcinogenic forms.

A second species, *Armandia brevis*, was obtained (a generous gift from Casimir Rice at NOAA/NMFS, Seattle, WA). This species burrows into and ingests sediment, thus ensuring good

exposure to potential contaminants. Upon exposure to [ $^{14}\text{C}$ ]-benzo[a]pyrene, the radiolabel was bound with the worms' DNA. The worms' life cycle is fairly well-characterized; they mature to breeding adults about 60 days after settling from the larval stage. The only drawbacks appear to be that they have a long larval stage (8 weeks), which requires micro algae as a food source, and they are a cold water species (10-15 °C, requiring a more elaborate setup for maintaining cultures).

A third species, *Neanthes succinea*, has recently been obtained. This worm is also a detritus-feeding, sediment-burrowing worm, but it has the benefit of having a wide temperature and salinity tolerance. This species has also been shown to metabolically activate [ $^{14}\text{C}$ ]-B[a]P in laboratory exposures. However, little is currently known about its life history and culture techniques. These will be investigated to provide a second species acceptable as a candidate for transformation.

**CONCLUSION:** To facilitate the performance of accurate risk assessments when they may be required, effective and clearly interpretable methods for assessing sediment genotoxicity are essential. Most of the presently available methods that rely on indicators in organisms not normally in intimate contact with sediments or in vitro methods applied to sediment extracts do not reflect realistic exposures. A few in vivo models employ appropriate species such as *Neanthes* (Pesche et al. 1981, 1985) or *Platynereis* (Jha et al. 1996). However, the former relies on SCE detection and the latter on chromosomal aberrations, both of which give limited information on the genotoxicity of the sediments as they may miss alterations such as base substitutions, deletions/additions, or frameshift mutations, all of which are acknowledged to play a large part in carcinogenesis.

A few in vivo fish models have successfully employed genotoxicity endpoints, but these models are more useful for monitoring contamination and remediation in situ than for sediments that are to be dredged and must be tested prior to disposal. Transgenic benthic, infaunal organisms solve these inadequacies and have the additional benefit of enabling determination of the nature of mutation that has occurred (addition/deletion, base substitution and the foci of mutation, i.e., the mutation spectrum). Additional benefits of this in vivo approach are the fact that one can potentially obtain information on acute toxicity (survival of the organism) and chronic toxicity (growth of the organisms during the exposure, reproductive viability) in the same organisms utilized for the genotoxicity assays.

During the final two years of the LEDO sediment genotoxicity work unit, attempts will be made as described in this Technical Note to produce a stable transgenic polychaete as a model for bioavailable genotoxicity in sediments, to characterize its responses to genotoxic sediments, and to provide cultures of the organism to other researchers in the field for further research, development, and application.

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- EEDP-09-4 Equipment Mobility in Confined Dredged Material Disposal Areas; Field Evaluations (January 1989)
- EEDP-09-5 Selecting Equipment for Use in Dredged Material Containment Areas (January 1990)
- EEDP-09-6 Alternative Dredging Equipment and Operational Methods to Minimize Sea Turtle Mortalities (December 1990)